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(54) Multi-specimen slides for immunohistologic procedures.

(57) A process for producing a slide bearing a spaced array of specimen fragments which comprises (i) cutting at least one specimen into a plurality of narrow strips; (ii) separating the plurality into groups of specimen strips; (iii) separately positioning strips from the groups in parallel grooves in a mold; (iv) embedding the strips in the mold in a first embedding medium to provide a structure comprising a base member having opposed first and second surfaces, the first surface being substantially planar; the second surface having ridges containing a specimen strip extending therefrom; (v) forming a stack of the structures with the terminal surface of the ridges of an upper structure abutting the substantially planar first surface of the next lower structure; the spaces between the ridges defining channels for receipt of a fluid; (vi) embedding the stack in a second embedding medium to form a block having a spaced array of parallel specimen strips embedded therein; the strips being so arranged that a section of the block includes a spaced array of cross-sections of each of the embedded specimen strips; (vii) dividing the block into sections each containing a spaced array of cross-sections of each of the embedded specimen strips; (viii) mounting at least one of such block sections on a slide.

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MULTI-SPECIMEN SLIDES FOR IMMUNOHISTOLOGIC PROCEDURES

BACKGROUND OF THE INVENTION

This invention relates to multi-specimen slides useful in immunohistologic procedures. More particularly, the invention relates to slides bearing a plurality of specimens in spaced array appropriate for automated image analysis and to technology germane to such slides.

Various multi-specimen slides are known. Paraffin block sections each containing multiple tissue specimens are described in Lillie, Histopathologic Technic and Practical Histochemistry, McGraw-Hill, Inc., New York, New York (1965) pp. 74-77. Composite snap-frozen tissue sections mounted on a slide for use in diagnostic autoimmunology are described in Nairn, Fluorescent Protein Tracing, 4th Ed., Churchill Livingstone, London (1976) pp. 131-138. Johnson, et al. Handbook of Experimental Immunology, 3rd Ed., Blackwell Scientific Publications, Oxford, England (1978) refers to composite frozen tissues useful for autoantibody testing with the admonition that "To get satisfactory sections the tissue pieces must be frozen together without leaving spaces between them..." (p. 154). Mason, et al. in Bullock, et al. Techniques in Immunocytochemistry, Vol. 2, Academic Press, London (1983) pp. 175-216 states that tissue culture supernatants may be tested again either paraffin embedded sections or cryostat sections of snap-frozen tissue. Cryostat sections may be placed in the wells of multitest slides (pp. 192-193). Mason also states that hybridoma supernatants may be tested on air dried cell smears (p. 192). Battifora describes a multitissue tumor block useful for immunohistochemical antibody testing in Laboratory Investigation 55:244-248 (1986). Various multitissue slides are described in Stocker U.S. patent 4,647,543.

Computer controlled automatic image analysis instruments useful with appropriate software to analyze the spaced specimen array of slides of this invention are commercially available. Typical instruments include Recognition Concepts, Inc, Gould DeAnza, Inc. and Megabesion, Inc.

SUMMARY OF THE INVENTION

This invention provides slides bearing a plurality of specimen fragments in spaced array appropriate for automated computer-controlled image analysis. The specimen fragments may be of any kind. Fixed or frozen unfixed tissue specimens and cell culture specimens are preferred. The invention also subsumes technology germane to the production and use of such slides.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation of a slide in accordance with the invention.

Figure 2 is a perspective view of a multiblade device for cutting specimens into strips.

Figure 3 is a perspective view of a mold provided with parallel grooves to receive specimen strips.

Figure 4 is a perspective view of an embedding medium structure having specimen strips containing ridges of a type formed from the mold of Figure 3.

Figure 5 is a perspective view of a stack of structures as shown in Figure 4.

Figure 6 is a perspective view of a container having perforated walls for receiving a stack of structures as depicted by Figure 5.

Figure 7 is a perspective view of a section as produced by a microtome or the like of a block as depicted in Figure 6.

DETAILED DESCRIPTION OF THE INVENTION

Slides pursuant to the invention bear a plurality of specimen fragments in a spaced array. The pattern of the array may be selected to accommodate computer controlled image analysis. Quadrangular, i.e., square or rectangular patterns are preferred.

The invention is particularly concerned with slides useful in immunohistologic procedures. Such slides typically have tissues or cell culture specimen fragments mounted thereon. Either fixed or unfixed, frozen tissue specimens may be used. For many purposes, frozen tissue slides are preferred to insure the preservation of substantially unmodified tissue components such as antigens. The tissue specimens may be stained in known manner.

Figure 1 illustrates a slide 10 bearing a plurality of tissue specimen fragments 11 in a substantially equally spaced rectangular array. In practice, the spacing may be arranged to accommodate automated image analysis. For example, a minimum of 3 pixels or about 75 to 100 microns space between specimens at a magnification of 25 times with a 512 x 512 array is appropriate.

Slides in accordance with the invention are

appropriately provided with fragments from a plurality of different relatively large tissue or cell culture specimens. Each relatively large specimen is cut into narrow strips in any appropriate manner, for example, with a multiblade cutting device as illustrated by Figure 2. Referring to the figure, the device comprises a series of blades 12 separated by spacer means 13 of an appropriate dimension to provide specimen strips of a desired narrow width. The cutting device knives and spacers are mounted on support means 14, each of which includes a removable retention means 15.

The relatively large tissue specimens for subdivision into narrow strips may be obtained from any available source such as autopsies or operations. Cell culture samples may, for example, be suspended in a gel, and the gel poured over a plate and dried to provide a layer of appropriate thickness, preferably about 0.5 to about 1.5 mm, and the layer thereafter removed from the plate and cut into narrow strips with a device as shown in Figure 2. Cell culture smears formed in known manner, see Mason, supra at page 193, comprise another source of specimen strips.

Strips 16 of fixed or of unfixed frozen tissue or of cell culture are placed in the parallel grooves 17 of a mold such as the mold 18 illustrated by Figure 3. An appropriate embedding medium, e.g., agar gel, is added to the mold containing the specimen strips and allowed to solidify thus producing a solidified embedding medium structure 19 as illustrated by Figure 4 upon removal from the mold 18.

The structure 19 comprises embedding medium in the form of a base member 20 having a substantially planar surface 21 and an opposed surface 22 having a plurality of spaced ridges 23 extending therefrom. Each ridge 23 includes a specimen strip 16.

A plurality of structures 19 are stacked as shown by Figure 5. In the stack, the terminal surfaces 24 of each ridge 23 abut the planar surface of the adjacent lower structure. The spaces between ridges provide channels 25 for access of fluids such as fixatives to the specimen strips in the ridges.

The stack of structures is placed in a container 26 as shown by Figure 6. The container walls include perforations 27 to permit the ingress and egress of fluids such as clearing and dehydrating agents.

A fixative may be introduced into and passed through the channels 25 to condition the specimen strips for further processing.

After fixing, the stack of structures 19 is removed from the container 26 and placed in a deep mold for final embedding to form a multispecimen block. The final embedding medium may be conventional, for example, paraffin or another wax, a high molecular weight polyethylene glycol or polyvinyl alcohol, nitrocellulose, a methacrylate resin, or an epoxy resin.

The block is sectioned by a microtome or like device to provide a plurality of sections 28, each containing a spaced array of specimen sections as shown in Figure 7. In the spaced array the channels 25 are filled by the final embedding material.

The block sections are mounted in known manner to provide slides of the kind indicated generally by the slide 10 of Figure 1.

To produce slides of the invention bearing fragments of unfixed frozen tissue or of frozen cell cultures, snap-frozen unfixed, preferably different, specimens are cut into narrow strips, placed while frozen in the parallel grooves 18 of a mold such as the mold 18, and embedded in an embedding medium such as OCT appropriate for use in freeze drying procedures to produce frozen structures 19 of the kind illustrated by Figure 4. Such structures, while frozen are stacked and the stack is embedded in a final embedding medium to provide a frozen block containing a plurality of spaced, parallel specimen strips as shown generally by Figure 7. The block is sectioned, e.g., by a cryostat to provide sections containing a plurality of specimen fragments in spaced array also as shown by Figure 7. The sections are mounted, in known manner, while frozen on slides and may thereafter be freeze dried.

Specimen fragments on the slides of this invention may be arranged in defined segments in which related specimen fragments are grouped together or associated in a manner to facilitate automated image processing. For example, one run of specimens, each of different, but known characteristics, may be positioned across a slide, e.g., a top run, to provide standards. Columns of unknown specimens may be provided above or below each standard included.

Claims

1. A process for producing a slide bearing a spaced array of specimen fragments which comprises:

(i) cutting at least one specimen into a plurality of narrow strips;

(ii) separating said plurality into groups of specimen strips;

(iii) separately positioning strips from said groups in parallel grooves in a mold;

(iv) embedding said strips in said mold in a first embedding medium to provide a structure comprising a base member having opposed first and second surfaces, said first surface being substantially planar;

said second surface having ridges containing a specimen strip extending therefrom;

(v) forming a stack of said structures with the terminal surface of said ridges of an upper structure abutting the substantially planar first surface of the next lower structure;
the spaces between said ridges defining channels for receipt of a fluid;

(vi) embedding said stack in a second embedding medium to form a block having a spaced array of parallel specimen strips embedded therein; said strips being so arranged that a section of said block includes a spaced array of cross-sections of each of said embedded specimen strips;

(vii) dividing said block into sections each containing a spaced array of cross-sections of each of said embedded specimen strips;

2. The process of claim 1 in which the specimen comprises a fixed tissue, a frozen unfixed tissue. or a cell culture.

3. The process of claim 1 in which

(i) the specimen is a tissue fixed for storage;

(ii) said first embedding medium is agar gel or gelatin;

(iii) the stack of structures formed in step (v) is placed in contact with a fixative which occupies the channels defined by the spaces between the ridges of said structures;

(iv) said second embedding medium is paraffin, polyethylene glycol, a methacrylate resin or an epoxy resin.

4. A process for producing a slide bearing a spaced array of unfixed frozen or freeze dried tissue specimens which comprises

(i) cutting unfixed, frozen tissue specimens into a plurality of narrow strips;

(ii) separating said plurality into groups of frozen strips;

(iii) separately positioning the strips from each of said groups in parallel grooves in a mold;

(iv) embedding said so positioned strips in said mold in a cryogenic embedding medium to provide a frozen structure comprising a base member having opposed first and second surfaces
said first surface being substantially planar;
said second surface having a plurality of ridges containing a specimen strip extending therefrom;

(v) forming a stack of said frozen structures with the terminal surface of said ridges of an upper stack abutting the planar surface of the next lower structure;

(vi) embedding the frozen stack in a cryogenic embedding medium to produce a frozen embedding medium block having a spaced array of parallel specimen strips embedded therein
said strips being so arranged that a section of said block includes a spaced array of cross-sections of each of said specimen strips;

(vii) dividing said frozen block into sections each containing a spaced array of frozen cross-sections of each of said strips;

(viii) mounting at least one of said frozen sections on a slide.

5. A slide bearing specimen fragments in a spaced array appropriate for automated image analysis.

6. A slide as bearing frozen, unfixed tissue fragments in a spaced array appropriate for automatic image analysis.

7. A slide as defined by claim 6 on which the tissue fragments are freeze dried.

8. A slide bearing fixed tissue fragments in a spaced array appropriate for automatic image analysis.

9. A structure comprising
a base member formed from an embedding medium;
said base member having opposed first and second surfaces
said first surface being substantially planar;
said second surface having a plurality of spaced parallel ridges extending therefrom; and
specimen strips in at least some of said ridges.

10. A structure as defined in claim 9 in which said specimen strips are strips of fixed tissue, frozen unfixed tissue or of a cell culture composition.

11. A structure as defined by claim 9 in which said specimen strips are strips of fixed tissue and the embedding medium is agar gel or gelatin.

12. A structure as defined by claim 9 in which said specimen strips are strips of frozen, unfixed tissue and the embedding medium is cryogenic.

13. A structure as defined by claim 9 or claim 10 in which said ridges have substantially planar terminal surfaces.

14. A stack of structures as defined by claim 9 or claim 10 in which
the terminal surfaces of the ridges or an upper structure in said stack abut the substantially planar first surface of the next lower stack;
the spaces between said ridges defining parallel channels.

15. A process for substantially simultaneously fixing a plurality of tissue specimens which comprises introducing a fixative into the channels in a stack of structures as defined by claim 14 to contact the tissue specimens present in the ridges of the structures comprising said stack.

16. An embedding medium block having a spaced array of specimen strips embedded therein, said strips being so arranged that a section of said block normal to the longitudinal axis of said strips includes a spaced array of cross-sections of each of said embedded strips.

17. A block as defined by claim 16 in which specimen strips comprise fixed tissue, unfixed frozen tissue or a cell culture composition.

18. A multispecimen slide comprising a row of different specimens of known characteristics and a plurality of unknown specimens positioned above or below at least one of the known specimens in said row to provide at least a column including one known specimen and a plurality of unknown specimens.

19. A multispecimen slide as defined in claim 18 in which said known and unknown specimens are specimens of a cell culture composition or a fixed tissue.

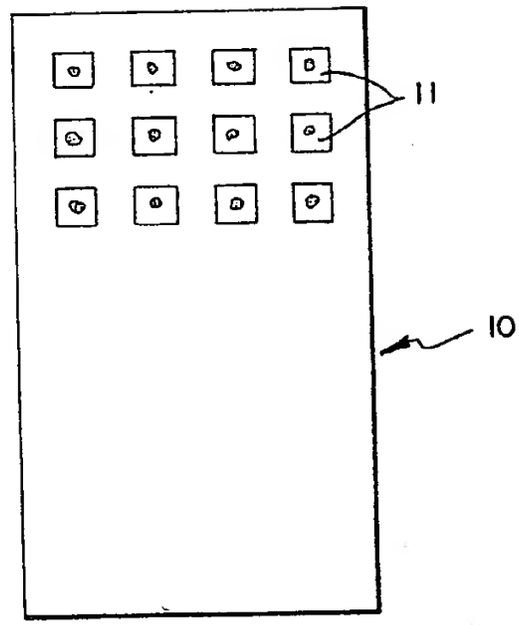


FIG. 1

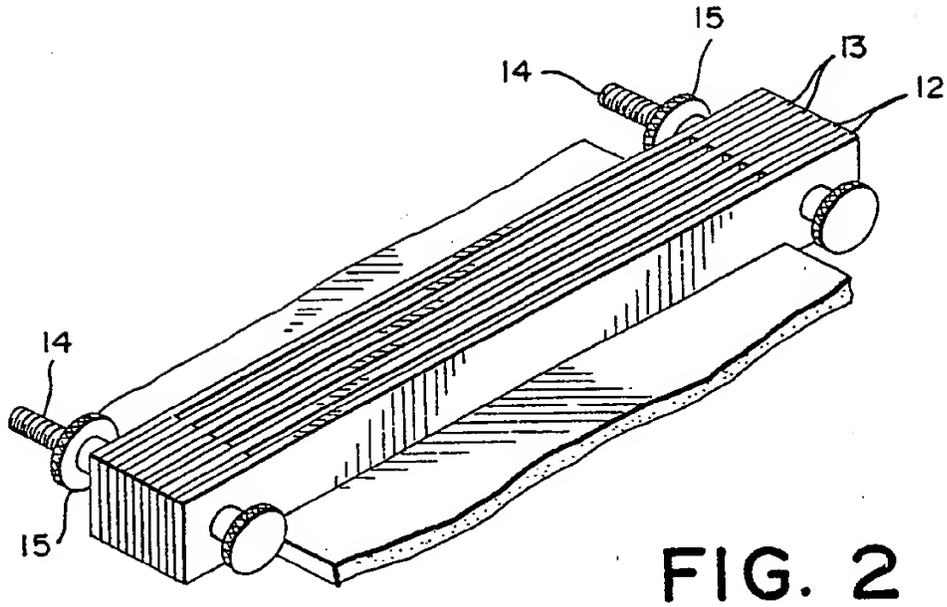


FIG. 2

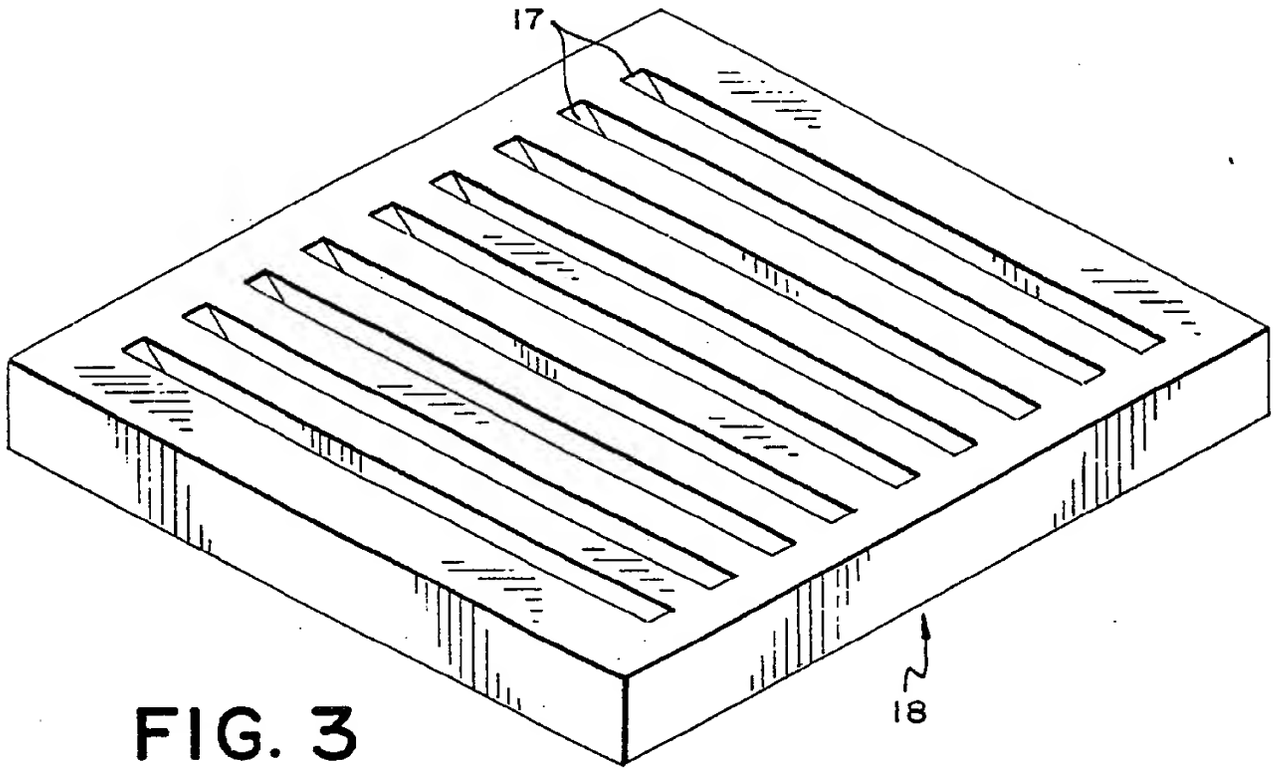


FIG. 3

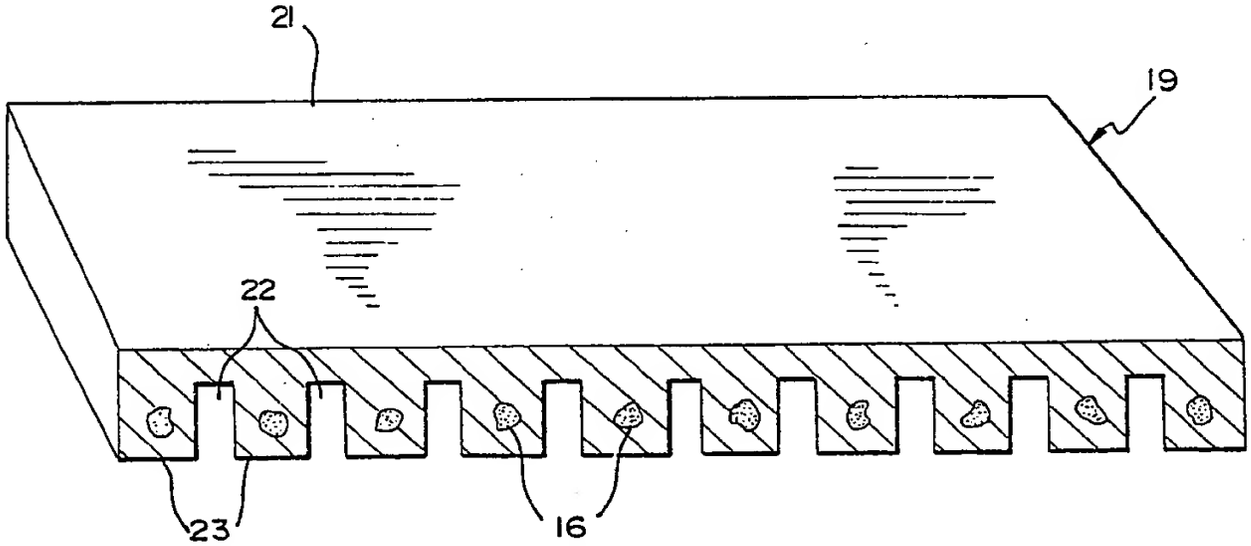


FIG. 4

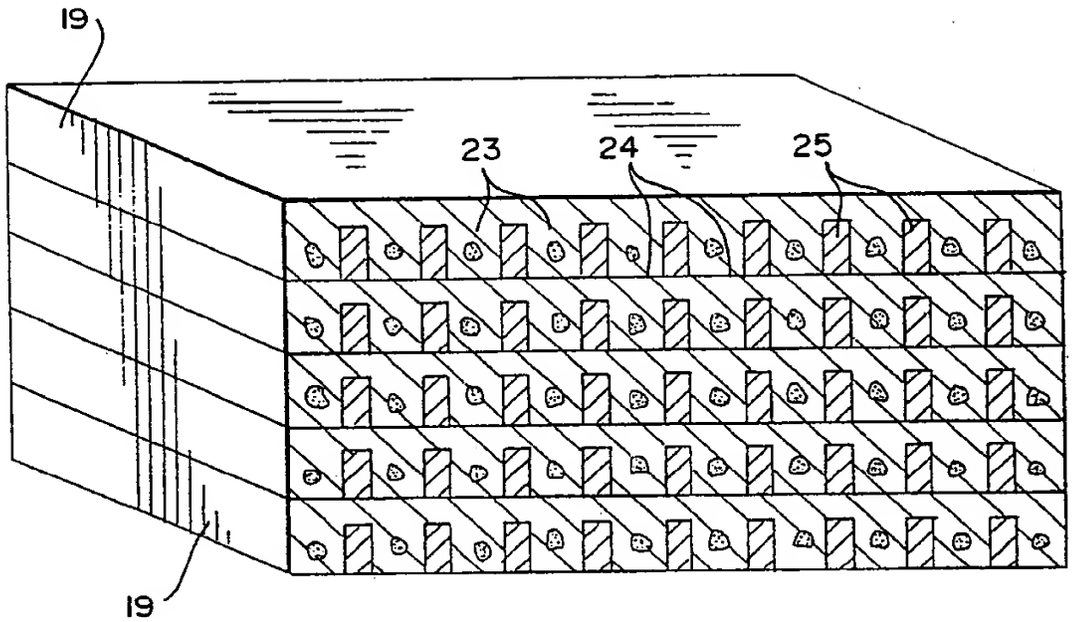


FIG. 5

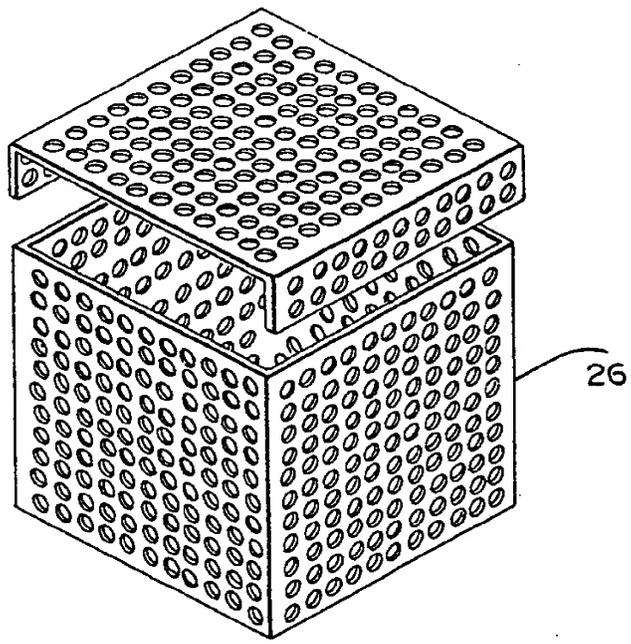
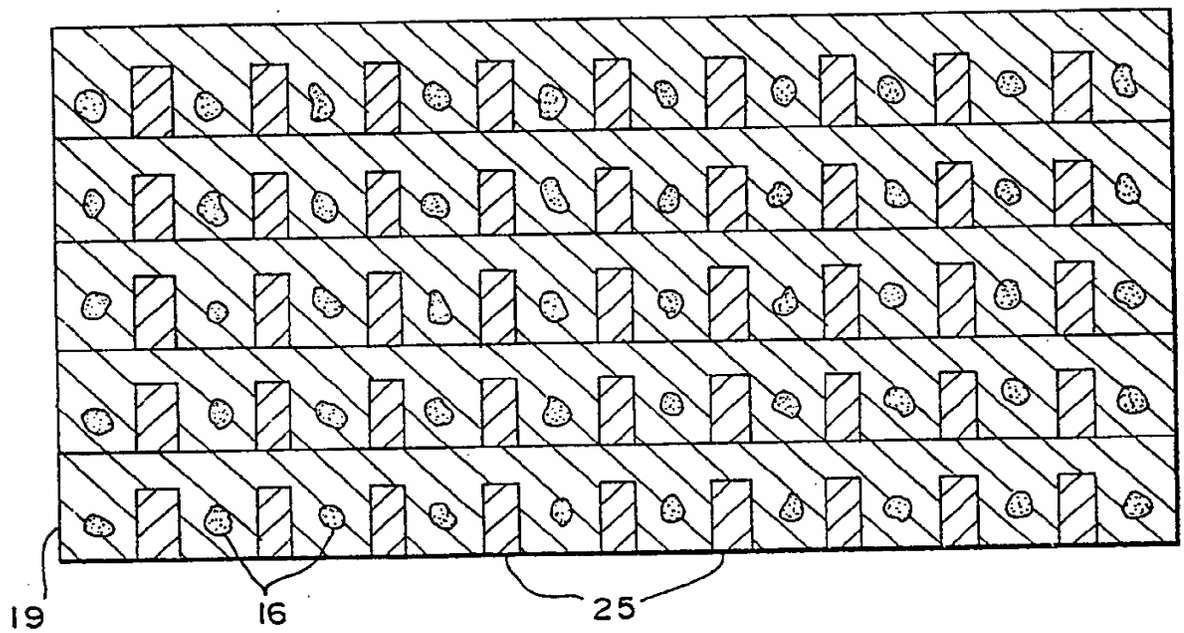


FIG. 6

FIG. 7



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European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 89 30 6458

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	EP-A-0 238 190 (BECKMAN RESEARCH INSTITUTE OF THE CITY OF HOPE) * Column 3, line 23 - column 4, line 35; column 5, lines 22-48 *	1	G 01 N 1/28
A,D	US-A-4 647 543 (W. STÖCKER)		

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

G 01 N

~~The present search report has been drawn up for all claims~~

Place of search	Date of completion of the search	Examiner
THE HAGUE	25-02-1991	HOCQUET A.P.E.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P0401)

European Patent Office

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid.

namely claims:

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

X LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions.

namely:

See sheet -B-

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid.

namely claims:

☒ None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims.

namely claims: 1-4,9-17

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions.

namely:

1. Claims 1-4,9-17: Process for producing a slide and structures obtained by this process.

2. Claims 5-8: Slide adapted for automatic image analysis.

3. Claims 18-19: Slide with two different portions for known and unknown specimens.